09/556,460

FILE 'CAPLUS' ENTERED AT 14:02:39 ON 12 APR 2002

=> S ENZYME;S BINDING;S SITE;S CYSTEINE;S SUBTILISIN;S TARGET
        638687 ENZYME
        351445 ENZYMES
L1   795314 ENZYME
               (ENZYME OR ENZYMES)

        707404 BINDING
          1610 BINDINGS
L2   707847 BINDING
               (BINDING OR BINDINGS)

        447356 SITE
        418972 SITES
L3   736987 SITE
               (SITE OR SITES)

         78936 CYSTEINE
          4258 CYSTEINES
L4   80633 CYSTEINE
               (CYSTEINE OR CYSTEINES)

          5434 SUBTILISIN
           403 SUBTILISINS
L5   5493 SUBTILISIN
               (SUBTILISIN OR SUBTILISINS)

        205680 TARGET
         72006 TARGETS
L6   248215 TARGET
               (TARGET OR TARGETS)

=> S L1 AND L2
L7   88561 L1 AND L2

=> S L1 AND L6
L8   17681 L1 AND L6

=> S L7 AND L8
L9   4481 L7 AND L8

=> S L9 AND L4
L10   250 L9 AND L4

=> S L10 AND L5
L11   3 L10 AND L5

=> D 1-3 CBIB ABS

L11 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2002 ACS
1996:483657 Document No. 125:134811 Production of C-terminal amidated peptides from recombinant protein constructs and therapeutic applications. Stout, Jay S.; Patridge, Bruce E.; Heriksen, Dennis B.; Holmquist, Barton; Wagner, Fred W. (Bionebraska, Inc., USA). PCT Int. Appl. WO 9617941 A2 19960613, 91 pp. DESIGNATED STATES: W: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US15799 19951207. PRIORITY: US 1994-350528 19941207.

AB A method for the prodn. of C-terminal amidated recombinant peptides is provided. The method employs a recombinant protein construct having multiple copies of a ***target*** peptide linked by intraconnecting peptides. The intraconnecting peptides permit the multicopy construct to be selectively reacted to produce product peptides having a C-terminal .alpha.-carboxamide. A recombinant gene contg. a DNA sequence coding for the recombinant protein construct and an expression cassette, an expression vector and a transformed cell including the recombinant gene are also provided.

L11 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2002 ACS
1995:924332 Mechanism-based ***cysteine*** protease inhibitors.. Palmer, J. T.; Rasnick, D.; Klaus, J. L. (Khepri Pharmaceuticals, South San Francisco, CA, 94080, USA). Book of Abstracts, 210th ACS National Meeting, Chicago, IL, August 20-24, Issue Pt. 2, MEDI-068. American Chemical Society: Washington, D. C. (English) 1995. CODEN: 61XGAC.
AB The upregulation of ***cysteine*** proteases has been implicated in a variety of disease states, including arthritis, cancer, and infectious diseases. Inhibitors therefore offer vast market potential as disease modifying agents. We have prepd. several series of mechanism-based inhibitors of human ***cysteine*** proteases (Cathepsins B, L, S, calpains I and II) and the parasitic protease cruzain. These compds. display second-order rate consts. of inactivation up to 56,000,000/M/s depending on the ***target*** . ***Cysteine*** protease inhibitors of these series bearing ***binding*** elements based on substrate specificity for the serine proteases chymotrypsin, elastase, and ***subtilisin*** neither inactivate nor bind to these ***enzymes*** . These inhibitors are chem. stable and are unreactive in the absence of the ***target*** proteases, as shown by their inertness toward circulating thiols such as glutathione.

L11 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2002 ACS
1989:434697 Document No. 111:34697 Computer designed stabilized proteins and method for producing same. Pantoliano, Michael W.; Ladner, Robert Charles (Genex Corp., USA). PCT Int. Appl. WO 8808164 A1 19881020, 66 pp. DESIGNATED STATES: W: DK, JP; RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1988-US849 19880318. PRIORITY: US 1987-34964 19870406.
AB A method for producing a protein having a potentially stabilizing disulfide bond comprises (1) comparing the distance between the centers-of-mass of 2 candidate ***target*** amino acid residues with the distance between the center-of-mass of the ***cysteine*** residues of a disulfide bond; (2) calcg. the error when the known disulfide bond is superimposed on the 2 candidate amino acid residues; (3) using the results of steps 1 and 2 to det. whether any such replacements can be made; and (4) producing the protein, forming the disulfide bond, and detg. the stability. The computerization of the process is described. The process was successfully applied to introduce stabilizing disulfide bonds into ***subtilisin*** BPN'.

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=> S L2(W)L3
L12     128815 L2(W)L3

=> S L1 AND L12
L13      22368 L1 AND L12

=> S L13 AND L4
L14       1247 L13 AND L4

=> S L5 AND L13
L15        201 L5 AND L13

=> S ANTAGONIST
        123677 ANTAGONIST
         87225 ANTAGONISTS
L16     164544 ANTAGONIST
                (ANTAGONIST OR ANTAGONISTS)

=> S L16 AND L15
L17          0 L16 AND L15

=> S L16 AND L7
L18       1489 L16 AND L7

=> S L16 AND L13
L19        338 L16 AND L13

=> S L19 AND L4
L20          2 L19 AND L4
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=> S L20 NOT L11
L21 2 L20 NOT L11

=> D 1-2 CBIB ABS

L21 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS
2000:92849 Document No. 133:12208 Basic aspects of selectivity of pantoprazole and its pharmacological actions. Beil, Winfried; Sewing, Karl-Friedrich; Kromer, Wolfgang (Institute of General Pharmacology, Hannover Medical School, Hannover, D-30625, Germany). Drugs of Today, 35(10), 753-764 (English) 1999. CODEN: MDACAP. ISSN: 0025-7656. Publisher: Prous Science.

AB A review with 69 refs. Pantoprazole sodium is a substituted benzimidazole deriv. which controls acid secretion by inhibition of gastric H+/K+-ATPase. The prodrug pantoprazole accumulates in the acidic space of the parietal cell where it is converted to the pharmacol. active principle, a thiophilic cyclic sulfenamide. The pH-dependent activation profile, i.e., activation at pH 1 vs. activation at pH 4-6, is more favorable for pantoprazole than for the other proton pump inhibitors (PPIs) currently available. In vitro, pantoprazole interferes less potently than omeprazole with biol. targets not related to gastric acid secretion. The gastric target sites for the pantoprazole sulfenamide are the ***cysteines*** 813 and 822 of the catalytic subunit of the H+/K+-ATPase. In contrast to the sites for omeprazole, the 2 ***binding*** ***sites*** of pantoprazole are located directly at the proton channel. In rats, dogs and humans, pantoprazole produces marked and prolonged inhibition of both basal and stimulated acid secretion. Overall, its antisecretory potency is equal to that of omeprazole. Antiulcer activity has been demonstrated for pantoprazole in 2 rat models. As seen with H2-receptor ***antagonists*** and other PPIs, pantoprazole causes an increase in serum gastrin concn. which reflects the degree of gastric acid inhibition. Pantoprazole is mainly metabolized by cytochrome P 450 3A4 and 2C19, but displays a lower affinity for these phase I cytochrome P 450 ***enzymes*** than omeprazole. In contrast to the latter, pantoprazole is further conjugated with sulfate by the hepatic phase II metab. These 2 differences may explain why pantoprazole does not interfere with the metab. of any other drug thus far tested in humans.

L21 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2002 ACS
1996:282935 Document No. 124:340529 RANTES and MCP-3 ***antagonists*** bind multiple chemokine receptors. Gong, Jiang-Hong; Uguccioni, Mariagrazia; Dewald, Beatrice; Baggiolini, Marco; Clark-Lewis, Ian (Biomed. Res. Cent., Univ. British Columbia, Vancouver, BC, V6T 1Z3, Can.). J. Biol. Chem., 271(18), 10521-10527 (English) 1996. CODEN: JBCHA3. ISSN: 0021-9258.

AB ***Antagonists*** of multiple chemokines could be more effective than inhibitors of specific chemokines for controlling cell migration and inflammation. To attempt to identify such ***antagonists*** we characterized a no. of truncated analogs of regulated on activation normal T cell expressed protein (RANTES), monocyte chemoattractant protein (MCP)-3, and MCP-1. On the basis of their ability to compete for binding of their parent chemokines, three analogs were selected for cross-reactivity studies: RANTES (9-68), MCP-3 (10-76), and MCP-1 (9-76). These analogs bound to THP-1 monocytic cells with dissocn. consts. that were within 4-6-fold of their native counterparts, but they did not promote detectable chemotaxis of THP-1 cells or ***enzyme*** release from purified human monocytes. The RANTES (9-68) analog competed for the binding and inhibited the activities of all three chemokines. In contrast, native RANTES was specific for RANTES ***binding*** ***sites*** . However, truncation of either MCP-1 or MCP-3 did not change their resp. binding specificity. MCP-3 and MCP-3 (10-76) competed for binding of all three labeled chemokines. MCP-1 (9-76) competed strongly for binding of labeled MCP-1, but only weakly for the other two labeled ligands and inhibited the activities induced by MCP-1 and MCP-3 but not RANTES. Although RANTES (9-68) and MCP-3 (10-76) inhibited all three chemokines, the RANTES analog was significantly more potent for RANTES-induced activity. The results indicate that NH2-terminal residues partly det. the receptor specificity of RANTES, and deletions within this region permit binding to multiple chemokine receptors. The findings suggest the feasibility of design of high affinity multi-specific CC

chemokine ***antagonists*** .

=> S L19 AND L5
L22 0 L19 AND L5

=> S CHIMERIC
28781 CHIMERIC
23 CHIMERICS
L23 28789 CHIMERIC
(CHIMERIC OR CHIMERICS)

=> S L23 AND L7
L24 1116 L23 AND L7

=> S L24 AND L12
L25 205 L24 AND L12

=> S L23 AND L13
L26 205 L23 AND L13

=> D L26 28,42,48,85,102,133,144,171,181,197 CBIB ABS

L26 ANSWER 28 OF 205 CAPLUS COPYRIGHT 2002 ACS
2001:137701 Document No. 135:13969 Design of a ***chimeric*** 3-methyl-1,2,3-triazene with mixed receptor tyrosine kinase and DNA damaging properties: a novel tumor targeting strategy. Matheson, Stephanie L.; McNamee, James; Jean-Claude, Bertrand J. (Cancer Drug Research Laboratory, Department of Medicine, Division of Medical Oncology, McGill University Health Centre/Royal Victoria Hospital, Montreal, QC, Can.). Journal of Pharmacology and Experimental Therapeutics, 296(3), 832-840 (English) 2001. CODEN: JPETAB. ISSN: 0022-3565. Publisher: American Society for Pharmacology and Experimental Therapeutics.
AB The mixed epidermal growth factor receptor (EGFR)-DNA targeting properties of SMA41, a 6-(3-methyl-1,2,3-triazen-1-yl)-4-anilinoquinazoline designed to release N4-m-tolyl-quinazoline-4,6-diamine henceforth referred to as SMA52 [an inhibitor of EGFR tyrosine kinase (TK)] and methyldiazonium (a DNA methylating species) were studied in the O6-methylguanine-DNA methyltransferase (MGMT)-proficient and high EGFR-expressing epidermoid carcinoma of the vulva cell line A431. The effects of SMA41 were compared with those of SMA52 alone, and temozolomide (TEM), a clin. prodrug of 5-(3-methyltriazen-1-yl)imidazole-4-carboxamide (MTIC) that is inactive in MGMT-proficient cells. The results showed that (1) the ***chimeric*** SMA41 could degrade in serum-contg. medium (t1/2 of .apprx.30 min) to generate, as predicted, the free inhibitor SMA52 as the most abundant metabolite (.apprx.81% yield); (2) in contrast to SMA52 alone, the ***chimeric*** SMA41 and TEM induced significant DNA damage in A431 cells after 30-min or 2-h drug exposures, as confirmed by alk. single-cell gel microelectrophoresis (comet) assay; (3) SMA41 showed 5-fold greater affinity for the ATP ***binding*** ***site*** of EGFR than independently synthesized SMA52 in an ***enzyme*** assay and blocked EGF-induced tyrosine phosphorylation and EGFR autophosphorylation in A431 cells in a dose-dependent manner; (4) these mixed targeting properties of SMA41, combined with its ability to be converted to another potent EGFR TK inhibitor (e.g., SMA52) by hydrolytic cleavage, translated into over 8-fold greater antiproliferative activity than TEM, which showed no EGFR targeting properties (IC50 competitive binding > 100 .mu.M); (5) under continuous drug exposure (3-6-day sulforhodamine and clonogenic assays), SMA41 was almost equipotent with SMA52; however, in a short 2-h drug exposure followed by incubation in drug-free media, SMA52 showed an almost complete loss of antiproliferative activity over the whole dose range. In contrast, SMA41 retained almost 100% of its activity, indicating a more sustained growth inhibitory activity. The results in toto suggest that the superior antiproliferative activity of SMA41 may be due to a combination of events assocd. with its binary EGFR TK and DNA targeting properties.

L26 ANSWER 42 OF 205 CAPLUS COPYRIGHT 2002 ACS
2000:680449 Document No. 134:14615 Requirements for double-strand cleavage by ***chimeric*** restriction ***enzymes*** with zinc finger DNA-recognition domains. Smith, Jeff; Bibikova, Marina; Whitby, Frank G.; Reddy, A. R.; Chandrasegaran, Srinivasan; Carroll, Dana (Department of

Environmental Health Sciences, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD, 21205, USA). Nucleic Acids Research, 28(17), 3361-3369 (English) 2000. CODEN: NARHAD. ISSN: 0305-1048. Publisher: Oxford University Press.

AB This study concerns ***chimeric*** restriction ***enzymes*** that are hybrids between a zinc finger DNA-binding domain and the non-specific DNA-cleavage domain from the natural restriction ***enzyme*** FokI. Because of the flexibility of DNA recognition by zinc fingers, these ***enzymes*** are potential tools for cleaving DNA at arbitrarily selected sequences. Efficient double-strand cleavage by the ***chimeric*** nucleases requires two ***binding*** ***sites*** in close proximity. When cuts were mapped on the DNA strands, it was found that they occur in pairs sepd. by -4 bp with a 5' overhang, as for native FokI. Furthermore, amino acid changes in the dimer interface of the cleavage domain abolished activity. These results reflect a requirement for dimerization of the cleavage domain. The dependence of cleavage efficiency on the distance between two inverted ***binding*** ***sites*** was detd. and both upper and lower limits were defined. Two different zinc finger combinations binding to non-identical sites also supported specific cleavage. Mol. modeling was employed to gain insight into the precise location of the cut sites. These results define requirements for effective targets of ***chimeric*** nucleases and will guide the design of novel specificities for directed DNA cleavage in vitro and in vivo.

L26 ANSWER 48 OF 205 CAPLUS COPYRIGHT 2002 ACS

2000:473120 Document No. 133:234396 Structural Analysis of a ***Chimeric*** Bacterial .alpha.-Amylase. High-Resolution Analysis of Native and Ligand Complexes. Brzozowski, Andrzej M.; Lawson, David M.; Turkenburg, Johan P.; Bisgaard-Frantzen, Henrik; Svendsen, Allan; Borchert, Torben V.; Dauter, Zbigniew; Wilson, Keith S.; Davies, Gideon J. (Department of Chemistry Structural Biology Laboratory, University of York, Heslington York, YO10 5DD, UK). Biochemistry, 39(31), 9099-9107 (English) 2000. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB Several ***chimeric*** .alpha.-amylase genes were constructed by an in vivo recombination technique from the Bacillus amyloliquefaciens and Bacillus licheniformis genes. One of the fusion amylases (hereafter BA2), consisting of residues 1-300 from B. amyloliquefaciens and 301-483 from B. licheniformis, has been extensively studied by X-ray crystallog. at resolns. between 2.2 and 1.7 .ANG.. The 3-dimensional structure of the native ***enzyme*** was solved by multiple isomorphous replacement, and refined at a resoln. of 1.7 .ANG.. It consists of 483 amino acids, organized similarly to the known B. lichiniformis .alpha.-amylase structure, but features 4 bound calcium ions. Two of these form part of a linear cluster of three ions, the central ion being attributed to sodium. This cluster lies at the junction of the A and B domains with one calcium of the cluster structurally equiv. to the major Ca2+ ***binding*** ***site*** of fungal .alpha.-amylases. The third calcium ion is found at the interface of the A and C domains. BA2 contains a fourth calcium site, not obsd. in the B. licheniformis .alpha.-amylase structure. It is found on the C domain where it bridges the two .beta.-sheets. Three acid residues (Glu261, Asp328, and Asp231) form an active site similar to that seen in other amylases. In the presence of TRIS buffer, a single mol. of TRIS occupies the -1 subsite of the ***enzyme*** where it is coordinated by the three active-center carboxylates. Kinetic data reveal that BA2 displays properties intermediate to those of its parents. Data for crystals soaked in maltooligosaccharides reveal the presence of a maltotriose ***binding*** ***site*** on the N-terminal face of the (.beta./.alpha.)8 barrel of the mol., not previously described for any .alpha.-amylase structure, the biol. function of which is unclear. Data for a complex soaked with the tetrasaccharide inhibitor acarbose, at 1.9 .ANG., reveal a decasaccharide moiety, spanning the -7 to +3 subsites of the ***enzyme*** . The unambiguous presence of three unsatd. rings in the 2H3 half-chair/2E envelope conformation, adjacent to three 6-deoxypyranose units, clearly demonstrates synthesis of this acarbose-derived decasaccharide by a two-step transglycosylation mechanism.

L26 ANSWER 85 OF 205 CAPLUS COPYRIGHT 2002 ACS

1998:298605 Document No. 129:13019 ***Chimeric*** restriction

enzyme . Gal4 fusion to FokI cleavage domain. Kim, Yang-Gyun; Smith, Jeff; Durgesha, Mysore; Chandrasegaran, Srinivasan (Department Environmental Health Sciences, School Medicine, Johns Hopkins University, Baltimore, MD, 21205, USA). Biol. Chem., 379(4/5), 489-495 (English) 1998. CODEN: BICHF3. ISSN: 1431-6730. Publisher: Walter de Gruyter & Co..

AB Gal4, a yeast protein, activates transcription of genes required for metab. of galactose and melibiose. It binds as a dimer to a consensus palindromic 17-base pair DNA sequence. It is a member of the third family of proteins that contain Zn-mediated peptide loops that interact specifically with nucleic acids. Gal4 has a very distinctive Zn coordination profile and mode of DNA-binding. The authors report the creation of a novel site-specific endonuclease by linking the N-terminal 147 amino acids of Gal4 to the cleavage domain of FokI endonuclease. The fusion protein is active and under optimal conditions, binds to a 17 bp consensus DNA site and cleaves near this site. As expected, the cleavage occurs on either side of the consensus ***binding*** ***site*** (s).

L26 ANSWER 102 OF 205 CAPLUS COPYRIGHT 2002 ACS

1997:121410 Document No. 126:128719 Increasing the turnover rates of catalytic antibodies by importing active sites and selecting antibodies with increased substrate binding. Wohlstadter, Jacob N. (Wohlstadter, Jacob N., USA). PCT Int. Appl. WO 9640960 A1 19961219, 53 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US9827 19960606. PRIORITY: US 1995-476135 19950607; US 1995-505528 19950607.

AB A method of increasing the catalytic effectiveness of catalytic antibodies (abzymes) simplifies the problem of optimizing the active site by importing a known active site and using the selection process to conc. on improving substrate binding and selection. The selection method uses transgenic animals carrying the abzyme expression construct to select for increased effectiveness after immunization with an antigenic substrate or transition state analog. Placement of the imported catalytic sequence or of substrate binding sequences can be used to manipulate the degree of variation allowed. If the coding sequence for the site is placed in a region subject to somatic mutation, such as the V-D-J region, it will be subject to variation. If the sequence is placed in a const. or framework region, it will be relatively unaffected by somatic events. Construction of lysozyme-mimicking mouse catalytic antibodies is described.

L26 ANSWER 133 OF 205 CAPLUS COPYRIGHT 2002 ACS

1994:663668 Document No. 121:263668 Targetting of cytotoxins using fusion proteins with cell-specific ligands. Epenetos, Agamemnon Antoniou; Spooner, Robert Anthony; Deonarain, Mahendra (Imperial Cancer Research Technology Ltd., UK). PCT Int. Appl. WO 9415644 A1 19940721, 113 pp. DESIGNATED STATES: W: GB, JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1994-GB87 19940117. PRIORITY: GB 1993-686 19930115.

AB Cytotoxins are delivered to the target cells by administering them as fusion proteins with cell-specific ligands with high avidity. The cytotoxic moieties may include a ***binding*** ***site*** for a small mol., may be streptavidin, a dimeric RNase or DNAse I. The target cell-specific moiety may have two or more ***binding*** ***sites*** for the target cell and may be an antibody, antibody fragment, or a single-chain antibody. The protein may also contain a mediator portion and a directly or indirectly cytotoxic portion in which the mediator portion recognizes a target cell-specific mol. and provides a means of indirect targeting. These fusion proteins may also be useful for cell agglutination, particularly hemagglutination. The construction of fusion proteins of a single-chain antibody to a 4-hydroxy nitrophenylacetyl hapten and streptavidin and RNAse without the loss of hapten binding activity is demonstrated.

L26 ANSWER 144 OF 205 CAPLUS COPYRIGHT 2002 ACS

1994:186109 Document No. 120:186109 ***Chimeric*** restriction endonuclease. Kim, Yang Gyun; Chandrasegaran, Srinivasan (Sch. Hyg.

Public Health, Johns Hopkins Univ., Baltimore, MD, 21205-2179, USA). Proc. Natl. Acad. Sci. U. S. A., 91(3), 883-7 (English) 1994. CODEN: PNASA6. ISSN: 0027-8424.

AB FokI restriction endonuclease recognizes the nonpalindormic pentadeoxyribonucleotide 5'-GGATG-3'.cntdot.5'-CATCC-3' in duplex DNA and cleaves 9 and 13 nt away from the recognition site. Recently, the authors reported the presence of two distinct and separable domains within this ***enzyme*** : one for the sequence-specific recognition of DNA (the DNA-binding domain) and the other for the endonuclease activity (the cleavage domain). Here, the authors report the construction of a ***chimeric*** restriction endonuclease by linking the Drosophila Ultrabithorax homeodomain to the cleavage domain (FN) of FokI restriction endonuclease. The hybrid ***enzyme*** , Ubx-FN, was purified, and its cleavage properties were characterized. The hybrid ***enzyme*** shows the same DAN sequence-binding preference as that of Ubx; as expected, it cleaves the DNA away from the recognition site. On the 5'-TTAATGGTT-3' strand the hybrid ***enzyme*** cleaves 3 nt away from the recognition site, whereas it cuts the complementary 5'-AACCATTAA-3' strand 8, 9, or 10 nt away from the ***binding*** ***site*** . Similarly engineered hybrid ***enzymes*** could be valuable tools in phys. mapping and sequencing of large eukaryotic genomes.

L26 ANSWER 171 OF 205 CAPLUS COPYRIGHT 2002 ACS

1992:229632 Document No. 116:229632 An Immunoglobulin fusion protein comprising a fibrin- ***binding*** ***site*** and a fibrinolytic ***enzyme*** . Haber, Edgar; Quertermous, Thomas (General Hospital Corp., USA). Eur. Pat. Appl. EP 478366 A2 19920401, 38 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1991-308855 19910927. PRIORITY: US 1990-589435 19900927.

AB A fusion protein comprising an antibody variable region with a fibrin- ***binding*** ***site*** and a fibrinolytic ***enzyme*** is prepd. by expression of the ***chimeric*** gene in a host cell. It can be used as an efficient thrombolytic agent, or a thrombus-detecting agent when the protein is further radioactively or paramagnetically labeled. Plasmid pSVD8T encoding the variable regions of monoclonal antibodies 59D8 specific to fibrin and the B chain of tissue-type plasminogen activator (t-PA) was prepd. and used for transformation of a hybridoma 59D8 variant, which are unable to produce ordinary heavy chains. The fusion protein purified from the transformants possessed 70% of the activity of the natural t-PA and 1/10 of the fibrin affinity of the natural antibody.

L26 ANSWER 181 OF 205 CAPLUS COPYRIGHT 2002 ACS

1991:404723 Document No. 115:4723 Diagnostic immunoassay using ***chimeric*** monoclonal antibodies. Kaluza, Brigitte; Lenz, Helmut (Boehringer Mannheim G.m.b.H., Fed. Rep. Ger.). Ger. Offen. DE 3900534 A1 19900712, 22 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1989-3900534 19890110.

AB A substance is detd. immunol. in blood or serum by binding to 2 monoclonal antibodies R1 and R2, where R1 is bound or able to bind to a solid phase, R2 is labeled, and .gtoreq.1 of R1 and R2 is a ***chimeric*** antibody or fragment thereof. Thus, sheep anti-mouse Fc.gamma. IgG was adsorbed onto microtiter plates, followed by Crotein C to sat. remaining ***binding*** ***sites*** . To the anti-Fc.gamma. was bound a ***chimeric*** monoclonal anti-TSH antibody of human origin prepd. by genetic engineering methods, in which the variable regions were replaced by the corresponding regions of a mouse antibody. Then the plate was incubated with samples contg. TSH, followed by a mouse monoclonal antibody to TSH conjugated to peroxidase, and finally by a peroxidase substrate (ABTS) for photometric detn. of TSH.

L26 ANSWER 197 OF 205 CAPLUS COPYRIGHT 2002 ACS

1989:569997 Document No. 111:169997 Insertion of new sequences into the catalytic domain of an ***enzyme*** . Starzyk, Ruth M.; Burbaum, Jonathan J.; Schimmel, Paul (Dep. Biol., Massachusetts Inst. Technol., Cambridge, MA, 02139, USA). Biochemistry, 28(21), 8479-84 (English) 1989. CODEN: BICHAW. ISSN: 0006-2960.

AB Activities of ***enzymes*** can be modified by the replacement of active-site amino acids with residues that strengthen specific interactions with substrates or that alter the specificity. The scope for

engineered ***enzymes*** would be broadened if addnl., new sequences could be inserted into a catalytic domain. Properly designed, these sequences could encode new ligand ***binding*** ***sites*** , be intermediates in construction of ***chimeric*** ***enzymes*** , or alter the internal flexibility and breathing modes of the active site region. As a 1st step towards this objective, oligopeptides of .ltoreq.14 amino acids were inserted into various locations within an 82-amino acid region of the adenylate synthesis domain of Escherichia coli methionyl-tRNA synthetase. These sites include ones that are flanked by sequences that are conserved between the proteins from E. coli and the yeast Saccharomyces cerevisiae and those that are essential for activity and stability. All of the insertional mutants are stable and some have catalytic parameters for adenylate synthesis that are comparable to those of the wild type ***enzyme*** .

```
=> E DAVIS B/AU
=> S E3,E12,E57,E62,E67
           117 "DAVIS B"/AU
             4 "DAVIS B G"/AU
            17 "DAVIS BEN"/AU
            16 "DAVIS BENJAMIN"/AU
            28 "DAVIS BENJAMIN G"/AU
L27         182 ("DAVIS B"/AU OR "DAVIS B G"/AU OR "DAVIS BEN"/AU OR "DAVIS
               BENJAMIN"/AU OR "DAVIS BENJAMIN G"/AU)

=> E JONES J/AU
=> S E3,E13,E14,E24
           139 "JONES J"/AU
           126 "JONES J B"/AU
            14 "JONES J B JR"/AU
             5 "JONES J BYRON"/AU
L28         284 ("JONES J"/AU OR "JONES J B"/AU OR "JONES J B JR"/AU OR "JONES
               J BYRON"/AU)

=> E JONES JOHN/AU
=> S E3,E8,E9,E14
            27 "JONES JOHN"/AU
             8 "JONES JOHN B"/AU
            12 "JONES JOHN B JR"/AU
            19 "JONES JOHN BRYAN"/AU
L29         66 ("JONES JOHN"/AU OR "JONES JOHN B"/AU OR "JONES JOHN B JR"/AU
               OR "JONES JOHN BRYAN"/AU)

=> E BOTT R/AU
=> S E3,E9,E17,E20,E21
           34 "BOTT R"/AU
            1 "BOTT R R"/AU
           24 "BOTT RICHARD"/AU
           22 "BOTT RICHARD R"/AU
            7 "BOTT RICHARD RAY"/AU
L30        88 ("BOTT R"/AU OR "BOTT R R"/AU OR "BOTT RICHARD"/AU OR "BOTT
              RICHARD R"/AU OR "BOTT RICHARD RAY"/AU)

=> E SANFORD K/AU
=> S E3,E8-E10
             2 "SANFORD K"/AU
             5 "SANFORD KARL"/AU
             6 "SANFORD KARL J"/AU
             6 "SANFORD KARL JOHN"/AU
L31         19 ("SANFORD K"/AU OR "SANFORD KARL"/AU OR "SANFORD KARL J"/AU OR
               "SANFORD KARL JOHN"/AU)

=> E ESTELL D/AU
=> S E4,E6-E9
             6 "ESTELL D A"/AU
             5 "ESTELL DAVID"/AU
            44 "ESTELL DAVID A"/AU
             7 "ESTELL DAVID AARON"/AU
             1 "ESTELL DAVID BARON"/AU
L32         63 ("ESTELL D A"/AU OR "ESTELL DAVID"/AU OR "ESTELL DAVID A"/AU OR
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"ESTELL DAVID AARON"/AU OR "ESTELL DAVID BARON"/AU)

=> S L27-L32
L33 679 (L27 OR L28 OR L29 OR L30 OR L31 OR L32)

=> S L27,L28,L29,L30,L31,L31
L34 627 (L27 OR L28 OR L29 OR L30 OR L31 OR L31)

=> S L27,L28,L29,L30,L31,L31,L32
L35 679 (L27 OR L28 OR L29 OR L30 OR L31 OR L31 OR L32)

=> S TARGETING
30272 TARGETING
2 TARGETINGS
L36 30272 TARGETING
(TARGETING OR TARGETINGS)

=> S L35 AND L36
L37 2 L35 AND L36

=> S L35 AND L13
L38 16 L35 AND L13

=> S L37,L38
L39 18 (L37 OR L38)

=> D 1-18 CBIB ABS

L39 ANSWER 1 OF 18 CAPLUS COPYRIGHT 2002 ACS
2001:781111 Document No. 135:340142 Prescreening of peptide libraries for non-specific binding in selection of target-specific ligands from a peptide library. ***Estell, David A.*** ; Murray, Christopher J.; Tijerina, Pilar; Chen, Yiyou (Genencor International, Inc., USA). PCT Int. Appl. WO 2001079479 A2 20011025, 51 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US11811 20010411. PRIORITY: US 2000-PV197259 20000414.
AB A selective ***targeting*** method is disclosed comprising contacting a library of ligands, particularly a peptide library, with an anti-target to allow the ligands to bind to the anti-target; sepg. the non-binding ligands from the anti-target bound ligands, contacting the non-binding anti-target ligands with a target allowing the unbound ligands to bind with the target to form a target-bound ligand complex; sepg. the target-bound ligand complex from ligands which do not bind to the target, and identifying the target-bound ligands on the target-bound ligand complex wherein the target-bound ligands have a KD in the range of about 10-7 to 10-10 M. Addnl. claimed are the ligands identified according to the method. A method of screening a peptide library for specific ligands that uses a preliminary counter selection for non-specific binding is described. The preliminary screen is against common matrixes for immobilization such as fabrics, ceramics, glass, stainless steel and plastic. Unbound peptides are then screened for specific binding to a preferred target. Preferably, the target-bound ligands have a KD in the range of about 10-7 to 10-10 M. The uses of the present invention extend to antibody epitope mapping, identifying new ligands for binding mols. like enzymes and hormone receptors, identifying new pesticides, drug development, identifying industrial catalysts, identifying in vivo and in vitro diagnostic agents, increasing efficiency of enzyme catalysts, controlling protease action in vivo, changing inhibitory properties of targeted proteins, developing a targeted enzyme, for selective delivery of gene therapy vectors to specific tissues or cell types and for use in drug delivery or targeted activities.

L39 ANSWER 2 OF 18 CAPLUS COPYRIGHT 2002 ACS
2001:331144 Document No. 135:153097 Glycosylation of the primary binding

pocket of a subtilisin protease causes a remarkable broadening in stereospecificity in peptide synthesis. Matsumoto, Kazutsugu; ***Davis,*** *** Benjamin G.*** ; Jones, J. Bryan (Department of Applied Chemistry and Biotechnology, Fauclty of Engineering, Fukui University, Fukui, 910-8507, Japan). Chemical Communications (Cambridge, United Kingdom) (10), 903-904 (English) 2001. CODEN: CHCOFS. ISSN: 1359-7345. Publisher: Royal Society of Chemistry.

AB Site-selective glycosylation at position 166 at the base of the primary specificity S1 pocket in the serine protease subtilisin Bacillus lentus (SBL) created glycoproteins that are capable of catalyzing the coupling reactions of not only L- amino acid esters but also D-amino acid esters to give the corresponding dipeptides in good yields. This result of greatly broadened substrate specificities can be rationalized by the interaction of the glycans acting as chiral auxiliaries in stereochem. mismatched pairs.

L39 ANSWER 3 OF 18 CAPLUS COPYRIGHT 2002 ACS

2000:772488 Document No. 133:340212 Specifically targeted catalytic antagonists and uses thereof. ***Davis, Benjamin G.*** ; ***Jones,*** *** John Bryan*** ; ***Bott, Richard R.*** ; ***Sanford, Karl John*** ; ***Estell, David Baron*** (Genencor International, Inc., USA). PCT Int. Appl. WO 2000064485 A2 20001102, 144 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US10988 20000421. PRIORITY: US 1999-PV131362 19990428.

AB The invention provides chimeric mols. that are catalytic antagonists of a target mol. The catalytic antagonists of this invention preferably comprise a ***targeting*** moiety attached to an enzyme that degrades the mol. specifically bound by the ***targeting*** moiety. The catalytic antagonists of this invention thus bind to a target recognized by the ***targeting*** moiety (e.g., a receptor) the enzyme component of the chimera then degrades all or part of the target. This typically results in a redn. or loss of activity of the target and release of the chimeric mol. The chimeric mol. is then free to attack and degrade another target mol. A mutant of subtilisin Bacillus lentus (SBL) at S156C site was modified with 4-(6-methanethiosulfonyl)hexylpyrazole (MTS-pyrazole), and examd. its ***targeting*** activity to horse liver alc. dehydrogenase (HLADH).

L39 ANSWER 4 OF 18 CAPLUS COPYRIGHT 2002 ACS

2000:441949 Document No. 133:70717 Chemical modification of ***enzymes*** with methanethiosulfonate reagents for adding multiple charges and altering specificity and/or activity. ***Davis, Benjamin G.*** ; ***Jones, John Bryan*** ; ***Bott, Richard R.*** (Genencor International, Inc., USA). PCT Int. Appl. WO 2000037658 A2 20000629, 93 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US30362 19991220. PRIORITY: US 1998-PV113130 19981221; US 1999-467536 19991220.

AB A method and reagent kit for altering specificity of ***enzymes*** by chem. modification using methanethiosulfonate reagents combined with genetic engineering based site-directed mutagenesis, is disclosed. One or more amino acid residues of ***enzymes*** are replaced by cysteine residues, where the cysteine residues are modified by replacing the thiol hydrogen in the cysteine residues with a substituent group providing a thiol side chain comprising a multiply charged moiety. Preferred ***enzymes*** are a serine hydrolase or a protease such as Bacillus lentus subtilisin, cellulase, amylase, lactase, or lipase, and the amino acid replacement occurs in the ***binding*** ***site*** . Preferred amino acids for replacement by cysteine are asparagine, leucine,

methionine, or serine. In case of a trypsin-type serine protease, it also includes tyrosine, and glutamine, and for alpha/beta serine hydrolase such as Candida antarctica lipase, threonine, valine, isoleucine, and alanine. Neg. charges can be introduced by sulfonatoethyl thiol, 4-carboxybutyl thiol, 3,5-dicarboxybenzyl thiol, 3,3-dicarboxybutyl thiol, and 3,3,4-tricarboxybutyl thiol. Pos. charges can be introduced by aminoethyl thiol, 2-(trimethylammonium)ethyl thiol, 4,4-bis(aminomethyl)-3-oxo-hexyl thiol, and 2,2-bis(aminomethyl)-3-aminopropyl thiol. Those multiply charges moiety may be either a dendrimer or a polymer. A method of assaying for a preferred ***enzyme*** and detg. the catalytic efficiency of an ***enzyme*** by detg. the degree of stain removal from the material is also claimed. Use of detergent as component of ***enzyme*** -contg. compn. is claimed.

L39 ANSWER 5 OF 18 CAPLUS COPYRIGHT 2002 ACS
2000:9420 Document No. 132:134059 The controlled introduction of multiple negative charge at single amino acid sites in subtilisin Bacillus lentus. ***Davis, Benjamin G.*** ; Shang, Xiao; DeSantis, Grace; ***Bott,*** *** Richard R.*** ; Jones, J. Bryan (Department of Chemistry, University of Toronto, Toronto, ON, M5S 3H6, Can.). Bioorganic & Medicinal Chemistry, 7(11), 2293-2301 (English) 1999. CODEN: BMECEP. ISSN: 0968-0896. Publisher: Elsevier Science Ltd..

AB The use of methanethiosulfonates as thiol-specific modifying reagents in the strategy of combined site-directed mutagenesis and chem. modification allows virtually unlimited opportunities for creating new protein surface environments. As a consequence of our interest in electrostatic manipulation as a means of tailoring ***enzyme*** activity and specificity, the authors have adopted this approach for the controlled incorporation of multiple neg. charges at single sites in the representative serine protease, subtilisin Bacillus lentus (SBL). A series of mono-, di- and triacidic acid methanethiosulfonates were synthesized and used to modify cysteine mutants of SBL at positions 62 in the S2 site, 156 and 166 in the S1 site and 217 in the S1' site. Kinetic parameters for these chem. modified mutant (CMM) ***enzymes*** were detd. at pH 8.6 under conditions which ensured complete ionization of the unnatural amino acid side-chains introduced. The presence of up to three neg. charges in the S1, S1' and S2 subsites of SBL resulted in up to 11-fold lowered activity, possibly due to interference with oxyanion stabilization of the transition state of the hydrolytic reactions catalyzed. Each unit increase in neg. charge resulted in a raising of Km and a redn. of kcat. However, no upper limit was obsd. for increases in Km, whereas decreases in kcat reached a limiting value. Comparison with sterically similar but uncharged CMMs revealed that electrostatic effects of neg. charges at positions 62, 156 and 217 are detrimental, but are beneficial at position 166. These results indicate that the ground-state binding of SBL to the std. substrate, Suc-AAPF-pNA, to SBL is reduced, but without drastic attenuation of catalytic efficiency, and show that SBL tolerates high levels of charge at single sites.

L39 ANSWER 6 OF 18 CAPLUS COPYRIGHT 2002 ACS
1999:598700 Document No. 131:348435 Altered Flexibility in the Substrate-***binding*** ***Site*** of Related Native and Engineered High-alkaline Bacillus subtilisins. Mulder, Frans A. A.; Schipper, Dick; ***Bott, Richard*** ; Boelens, Rolf (Bijvoet Center for Biomolecular Research, Utrecht University, Utrecht, 3584 CH, Neth.). J. Mol. Biol., 292(1), 111-123 (English) 1999. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Academic Press.

AB High-alk. serine proteases have been successfully applied as protein degrading components of detergent formulations and are subject to extensive protein engineering efforts to improve their stability and performance. Dynamics has been suggested to play an important role in detg. ***enzyme*** activity and specificity and it is therefore of interest to establish how local changes in internal mobility affect protein stability, specificity and performance. Here we present the dynamic properties of the 269 residue serine proteases subtilisin PB92 (MaxacalTM) and subtilisin BLS (SavinaseTM), secreted by Bacillus lentus, and an engineered quadruple variant, DSAI, that has improved washing performance. T1, T2 and heteronuclear NOE measurements of the15N nuclei indicate that for all three proteins the majority of the backbone is very rigid, with only a limited no. of residues being involved in local mobility. Many of the residues that constitute the S1 and S4 pockets,

detg. substrate specificity, are flexible in soln. In contrast, the backbone amides of the residues that constitute the catalytic triad do not exhibit any motion. Subtilisins PB92, BLS and DSAI demonstrate similar but not identical NMR relaxation rates. A detailed anal. of local flexibility indicates that the motion of residues Thr143 and Ala194 becomes more restricted in subtilisin BLS and DSAI. Noteworthy, the loop regions involved in substrate binding become more structured in the engineered variant as compared with the two native proteases, suggesting a relation between altered dynamics and performance. Similar conclusions have been established by X-ray crystallog. methods, as shown in the accompanying paper. (c) 1999 Academic Press.

L39 ANSWER 7 OF 18 CAPLUS COPYRIGHT 2002 ACS
1999:598699 Document No. 131:334013 Engineered Bacillus lentus Subtilisins Having Altered Flexibility. Graycar, Thomas; Knapp, Mark; Ganshaw, Grant; Dauberman, Judy; ***Bott, Richard*** (Genencor International, Palo Alto, CA, 94304, USA). J. Mol. Biol., 292(1), 97-109 (English) 1999. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Academic Press.
AB The three-dimensional structures of engineered variants of Bacillus lentus subtilisin having increased enzymic activity, K27R/N87S/V104Y/N123S/T274A (RSYSA) and N76D/N87S/S103A/V104I (DSAI), were detd. by X-ray crystallog. In addn. to identifying changes in at. position we report a method that identifies protein segments having altered flexibility. The method utilizes a statistical anal. of variance to delineate main-chain temp. factors that represent significant departures from the overall variance between equiv. regions seen throughout the structure. This method reveals changes in main-chain mobility in both variants. Residues 125-127 have increased mobility in the RSYSA variant while residues 100-104 have decreased mobility in the DSAI variant. These segments are located at the substrate- ***binding*** ***site*** and changes in their mobility are believed to relate to the obsd. changes in proteolytic activity. The effect of altered crystal lattice contacts on segment flexibility becomes apparent when identical variants, detd. in two crystal forms, are compared with the native ***enzyme*** . (c) 1999 Academic Press.

L39 ANSWER 8 OF 18 CAPLUS COPYRIGHT 2002 ACS
1997:757129 Document No. 128:31836 Modified .alpha.-amylases having altered calcium binding properties. ***Bott, Richard R.*** ; Shaw, Andrew (Genencor International, Inc., USA). PCT Int. Appl. WO 9743424 A1 19971120, 30 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1997-US7609 19970506. PRIORITY: US 1996-645971 19960514.
AB Novel .alpha.-amylase ***enzymes*** are disclosed in which a new calcium ***binding*** ***site*** is modified by chem. or genetically altering residues assocd. with that calcium ***binding*** ***site*** . Thus, Bacillus licheniformis .alpha.-amylase crystals with space group P212121 and a = 118.3 .ANG., b = 119.0 .ANG. and c = 84.9 .ANG. are analyzed to form a model with an R-factor of 0.19 which allows detection of amino acid residues for potential modification. Modified B. licheniformis .alpha.-amylase may comprise a substitution at one or more of Q291, Q298, G299, G301, Y302, M304, L307, N309, Q340, F343, F403, H405, H406, D407, V409, G410, L427, I428, D430, G433, K436, N473, G474, and G475. Further substitutions or deletions may occur at one or more residues equiv. to M15, V128, H133, W138, N188, A209, and/or M197. The novel .alpha.-amylases have altered performance characteristics, such as low pH starch hydrolysis performance, stability, and activity profiles, and thus be of value for starch liquefication or in detergents.

L39 ANSWER 9 OF 18 CAPLUS COPYRIGHT 2002 ACS
1994:26197 Document No. 120:26197 Protein and solvent engineering of subtilisin BPN' in nearly anhydrous organic media. Wangikar, Pramod P.; Graycar, Thomas P.; ***Estell, David A.*** ; Clark, Douglas S.; Dordick, Jonathan S. (Cent. Biocatal. Bioprocess., Univ. Iowa, Iowa City, IA, 52242, USA). J. Am. Chem. Soc., 115(26), 12231-7 (English) 1993. CODEN: JACSAT. ISSN: 0002-7863.
AB The combined effects of protein and solvent engineering have been studied using subtilisin BPN' as a model protease. The effects of site-specific

mutations in the active site of subtilisin BPN' on the reactivity and substrate specificity of the ***enzyme*** are strongly dependent on the polarity of the substrate, active-site mutation, and solvent. In going from a polar solvent such as acetone to a nonpolar solvent such as hexane, subtilisin BPN' catalysis is activated dramatically (up to 178-fold) by employing a polar active-site mutation (Gly166 .fwdarw. Asn). This activation is proposed to be due to significant transition-state stabilization afforded by the polar mutation on subtilisin catalysis. Anal. of the individual kinetic and binding consts. for subtilisin indicates that the polar mutation in the S1 ***binding*** ***site*** of the ***enzyme*** results in improved catalysis over the wild-type solely because of increased ***enzyme*** -substrate interaction (decreased (Km)true). Water also effects the kinetics of subtilisin catalysis. In dry THF, acylation is rate limiting. Addn. of small concns. of water to the org. solvent (<2% vol./vol.) results in both an increased rate const. for acylation and a decreased (Km)true. At 2% (vol./vol.) added water and above, subtilisin reverts to a deacylation rate-limiting reaction on its ester substrates. These results suggest that water and polar mutations activate ***enzyme*** catalysis in nearly anhyd. solvents, albeit by different mechanisms, and further increase the general understanding of the nature of polarity on ***enzyme*** function. From a practical standpoint, it is concluded that the effectiveness of protein engineering is strongly dependent on the solvent conditions.

L39 ANSWER 10 OF 18 CAPLUS COPYRIGHT 2002 ACS

1991:58106 Document No. 114:58106 Alteration of the specificity of subtilisin BPN' by site-directed mutagenesis in its S1 and S1' ***binding*** ***sites*** . Bonneau, Pierre R.; Graycar, Thomas P.; ***Estell, David A.*** ; Jones, J. Bryan (Dep. Chem., Univ. Toronto, Toronto, ON, M5S 1A1, Can.). J. Am. Chem. Soc., 113(3), 1026-30 (English) 1991. CODEN: JACSAT. ISSN: 0002-7863.

AB The potential of site-directed mutagenesis as a means of controlled alteration of the substrate specificity of subtilisin BPN', and for differentiating between amide and ester substrates, has been investigated at both the S1 and S1' sites. The hydrophobic environment of the S1 ***binding*** ***site*** , for which Gly166 is at the bottom in the wild-type ***enzyme*** , is inhospitable to polar amino acid side chains such as that of N-tosyl-L-arginine Me ester (TAME). This can be partially compensated for by replacing Gly166 by amino acid residues, such as asparagine and serine (Ser), capable of H bonding with the guanidinium residue of TAME. In wild-type subtilisin BPN', the Tyr217 located near the end of the S1' leaving group site restricts somewhat the binding of the p-nitroanilide (pna) function of the tetrapeptide substrate succinyl-AAPFpna, for which acylation is the rate-detg. step in its subtilisin BPN'-catalyzed hydrolysis. Replacement of Tyr217 by the smaller amino acid residue leucine (Leu) permits the pna group to be better accomondated at S1'. In contrast, the Tyr217.fwdarw.Leu mutation is without significant effect on kcat for ester substrates, such as TAME or the thiobenzyl (tbe) ester analog of succinyl-AAPFpna, presumably because the methoxy or the leaving groups have departed prior to the deacylation rate-detg. step for such ester hydrolyses. Met222.fwdarw.Phe mutation reduces the vol. of the S1' pocket. In this case, the consequence is a redn. in amide hydrolysis rate without affecting catalysis of esters. This mutant is thus an excellent candidate for preparative-scale peptide synthesis applications. Triple replacement of the 156, 169, and 217 amino acid residues of subtilisin BPN' by those of subtilisin Carlsberg was shown previously to confer Carlsberg-like properties on the BPN' Glu156.fwdarw.Ser, Gly169.fwdarw.alanine, Tyr217.fwdarw.Leu mutant for amide hydrolyses. However, the Carlsberg-like properties of this triple mutant are not manifest for ester substrates.

L39 ANSWER 11 OF 18 CAPLUS COPYRIGHT 2002 ACS

1988:607529 Document No. 109:207529 Preparation of ***enzymes*** having altered transesterification/hydrolysis rate ratios and nucleophile specificities. Arbige, Michael Vincent; ***Estell, David Aaron*** ; Pepsin, Michael Jay; Poulose, Ayrookaran Joseph (Genencor, Inc., USA). Eur. Pat. Appl. EP 260105 A2 19880316, 11 pp. DESIGNATED STATES: R: DE, ES, FR, GB, NL, SE. (English). CODEN: EPXXDW. APPLICATION: EP 1987-307918 19870908. PRIORITY: US 1986-905363 19860909; US 1987-86869

19870821.

AB ***Enzymes*** with catalytic triads, e.g. serine or cystine hydrolases, are altered to change the transesterification rate/hydrolysis rate ratio and nucleophile specificity by altering amino acid residues within 15 .ANG. of the triad. [Phe-222]subtilisin BPN' has a transesterification/hydrolysis ratio (tyrosine Et ester substrate) 2.6-fold greater than that of the wild-type ***enzyme*** . When used to hydrolyze triacetin, this mutant showed a distinct preference for EtOH over H2O as the attacking nucleophile.

L39 ANSWER 12 OF 18 CAPLUS COPYRIGHT 2002 ACS

1988:545194 Document No. 109:145194 On the evolution of specificity and catalysis in subtilisin. Wells, J. A.; Cunningham, B. C.; Braycar, T. P.; ***Estell, D. A.*** ; Carter, P. (Dep. Biomol. Chem., Genetech Inc., South San Francisco, CA, 94080, USA). Cold Spring Harbor Symp. Quant. Biol., 52(Evol. Catal. Funct.), 647-52 (English) 1987. CODEN: CSHSAZ. ISSN: 0091-7451.

AB Serine proteases are characterized by a set of 3 catalytic residues consisting of a serine, a histidine, and an aspartic acid (the catalytic triad). Nature has apparently evolved the catalytic triad structure and the resulting serine protease mechanism at least twice. In particular, subtilisin (from species of Bacillus) and chymotrypsin (from mammalian pancreas) are genetically unrelated and their corresponding tertiary structures are entirely different. However, it is possible to virtually superimpose the catalytic triad and some features of the substrate- ***binding*** ***site*** from these convergently related ***enzymes*** . Here, 2 strategies for altering the substrate specificity of subtilisin are discussed. Previously, the substrate specificity of subtilisin has been altered by changing ***enzyme*** residues capable of making direct contact with a bound substrate. By appropriate amino acid substitutions it has been possible to sterically exclude binding of large substrates, promote binding of hydrophobic substrates, or enhance binding of charged substrates. Extending this approach, the degree to which the specificity properties of 2 functionally and evolutionarily divergent subtilisins can be switched by exchange of substrate contact residues is shown. These key substitutions represent <4% of the sequence differences between the 2 natural variant ***enzymes*** studied. Thus, mutation of substrate contact residues provides a powerful mechanism for divergence of substrate specificity within this homologous ***enzyme*** family. In the 2nd approach, termed substrate-assisted catalysis, the catalytic histidine is removed and its catalytic function is restored partially by a histidine side-chain from a bound substrate. In this case, substrates are distinguished at the level of catalysis by virtue of their ability to supply the missing catalytic function. Substrate-assisted catalysis is proposed as a possible evolutionary mechanism for installing the histidine in the catalytic triad to help explain the convergent evolution of the 2 serine protease families. Thus, modification of substrate binding contact residues provides a mechanism for the evolution of diverse substrate specificities. In addn., substrate-assisted catalysis is a potential means for ancestral subtilisin to have introduced the catalytic histidine and may help to account for the convergent evolution of serine proteases.

L39 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2002 ACS

1988:469443 Document No. 109:69443 The three-dimensional structure of Bacillus amyloliquefaciens subtilisin at 1.8 .ANG. and an analysis of the structural consequences of peroxide inactivation. ***Bott, Richard*** ; Ultsch, Mark; Kossiakoff, Anthony; Graycar, Thomas; Katz, Bradley; Power, Scott (Dep. Biomol. Chem., Genentech, Inc., South San Francisco, CA, 94080, USA). J. Biol. Chem., 263(16), 7895-906 (English) 1988. CODEN: JBCHA3. ISSN: 0021-9258.

AB The 3-dimensional structure of the subtilisin (I) from B. amyloliquefaciens (BAS) was refined to 1.8 .ANG. using the amino acid sequence deduced from the DNA coding sequence. The structure was essentially the same as the previously reported structures of I BPN' and I Novo detd. in different crystal forms, at 2.5 and 2.8 .ANG. resoln., resp. The largest differences in the 3 crystallog. models were seen in regions where the amino acid sequence used in the fit to the electron d. maps of BPN' and Novo differed from the gene sequence of BAS. The refined BAS model showed new features of cation binding, H-bonding, and internal solvent structure. The refined BAS model served as a basis for the anal.

of stereochem. factors involved in the peroxide inactivation of the ***enzyme*** . Methionine-222, which is adjacent to the catalytic serine-221, was quant. oxidized to the sulfoxide by H2O2 as had been previously shown for the related Bacillus licheniformis I. In addn. to this modification, partial to full oxidn. of 2 of the 4 remaining methionines was obsd. The oxidn. of the methionines did not correlate well with their solvent accessibility calcd. from the x-ray structure coordinates; in addn., only 1 of the 2 possible stereoisomers of methionine sulfoxide was formed. H2O2-induced modification of the OH groups of 2 tyrosines was also detected. Modeling suggested that most of the obsd. effect of oxidn. on the ***enzyme*** catalytic efficiency can be attributed to unfavorable interactions at the oxyanion ***binding*** ***site*** between the sulfoxide group at position 222 and the carbonyl O atom of the scissile peptide bond of the bound substrate.

L39 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2002 ACS

1988:18427 Document No. 108:18427 Recruitment of substrate-specificity properties from one ***enzyme*** into a related one by protein engineering. Wells, James A.; Cunningham, Brian C.; Graycar, Thomas P.; ***Estell, David A.*** (Dep. Biomol. Chem., Genentech, Inc., South San Francisco, CA, 94080, USA). Proc. Natl. Acad. Sci. U. S. A., 84(15), 5167-71 (English) 1987. CODEN: PNASA6. ISSN: 0027-8424.

AB The Bacillus licheniformis and B. amyloliquefaciens subtilisins differ by 31% in protein sequence and by factors of >60 in catalytic efficiency, kcat/Km (kcat = catalytic rate const.), toward various substrates. Despite large differences in sequence and substrate specificity for these serine proteases, only 2 amino acid substitutions (residues 156 and 217) occur within 4 .ANG. (contact distance) of modeled substrates, and a 3rd substitution (residue 169) is within 7 .ANG.. The 3 B. licheniformis substitutions (serine-156/alanine-169/leucine-217) were introduced into the wild-type B. amyloliquefaciens subtilisin (glutamate-156/glycine-169/tyrosine-217) by site-directed mutagenesis. The substrate specificity of the triple mutant approaches that of B. licheniformis ***enzyme*** when assayed with 7 different substrates that vary in charge, size, and hydrophobicity. Thus, specificity properties of distantly related and functionally divergent ***enzymes*** can be exchanged by limited amino acid replacements, in this case representing <4% of the sequence differences.

L39 ANSWER 15 OF 18 CAPLUS COPYRIGHT 2002 ACS

1987:549931 Document No. 107:149931 Purification and characterization of 2,5-diketo-D-gluconate reductase from Corynebacterium sp. Miller, Jeffrey V.; ***Estell, David A.*** ; Lazarus, Robert A. (Biocatal. Dep., Genentech, Inc., South San Francisco, CA, 94080, USA). J. Biol. Chem., 262(19), 9016-20 (English) 1987. CODEN: JBCHA3. ISSN: 0021-9258.

AB 2,5-Diketo-D-gluconate reductase, a novel ***enzyme*** that catalyzes the stereospecific NADPH-dependent redn. of 2,5-diketo-D-gluconate to 2-keto-L-gulonate, was purified to homogeneity by sequential anion exchange, Cibacron blue F3GA affinity, and gel permeation chromatog. from Corynebacterium ATCC 31090. Mol. wt. of the native form, detd. by gel permeation chromatog., is 35,000. The subunit mol. wt., detd. by SDS-PAGE is 34,000; thus, the ***enzyme*** is active as a monomer. A pI value of 4.4 is measured for the ***enzyme*** . N-terminal sequences are consistent with that predicted by the DNA sequence of the reductase gene. At 25.degree., pH 6.4, the turnover no. is 500 min-1, and the apparent Km values for 2,5-diketo-D-gluconate and NADPH are 26 mM and 10 .mu.M, resp. The ***enzyme*** is specific for NADPH, but the sugar ***binding*** ***site*** will also accept 5-keto-D-fructose and dihydroxyacetone as substrates. The ***enzyme*** is active over a broad pH range (pH 5-8) for the redn. of 2,5-diketo-D-gluconate; a sharp optimum at pH 9.2 is obsd. for the oxidn. of 2-keto-L-gulonate. An equil. const. value of 5.6 .times. 10-13M indicates that redn. of substrate by NADPH is highly preferred. An activation energy of 12.3 kcal mol-1 is measured. ***Enzyme*** turnover is slow relative to dehydration of the gem-diol at C-5 of the substrate.

L39 ANSWER 16 OF 18 CAPLUS COPYRIGHT 2002 ACS

1987:435629 Document No. 107:35629 Designing substrate specificity by protein engineering of electrostatic interactions. Wells, James A.; Powers, David B.; ***Bott, Richard R.*** ; Graycar, Thomas P.;

Estell, David A. (Dep. Biocatal., Genentech, Inc., South San Francisco, CA, 94080, USA). Proc. Natl. Acad. Sci. U. S. A., 84(5), 1219-23 (English) 1987. CODEN: PNASA6. ISSN: 0027-8424.

AB Protein engineering of electrostatic interactions between charged substrates and complementary charged amino acids, at 2 different sites in the substrate binding cleft of the protease subtilisin BPN', increases kcat/Km (kcat = catalytic rate const.) toward complementary charged substrates (.ltoreq.1900 times) and decreases kcat/Km toward similarly charged substrates. From kinetic anal. of 16 mutants of subtilisin and the wild type, the av. free energies for ***enzyme*** -substrate ion-pair interactions at the 2 different sites are calcd. to be -1.8 and -2.3 kcal/mol (1 cal = 4.18 J) [at 25.degree. in 0.1M Tris-HCl (pH 8.6)]. The combined electrostatic effects are roughly additive. These studies demonstrate the feasibility for rational design of charged ligand ***binding*** ***sites*** in proteins by tailoring of electrostatic interactions.

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1987:402858 Document No. 107:2858 Importance of conformational variability in protein engineering of subtilisin. ***Bott, Richard*** ; Ultsch, Mark; Wells, James; Powers, David; Burdick, Daniel; Struble, Martin; Burnier, John; ***Estell, David*** ; Miller, Jeffrey; et al. (Genentech, Inc., San Francisco, CA, 94080, USA). ACS Symp. Ser., 334(Biotechnol. Agric. Chem.), 139-47 (English) 1987. CODEN: ACSMC8. ISSN: 0097-6156.

AB The functional and structural effects were analyzed of single amino acid substitutions at position 166, where a glycine is found in all the subtilisins from a no. of Bacillus species. Substitutions of asparagine or lysine for glycine gave variant ***enzymes*** with high sp. activity or altered specificity, resp. The 3-dimensional structure of B. amyloliquefaciens subtilisin, originally detd. at 2.5 .ANG. resoln., was refined at 1.8 .ANG. resoln. The 3-dimensional structures of the asparagine and lysine 166 variants showed a pattern of localized structural perturbations rather than of global conformational change. The alterations of specificity in these variants appears to be linked to the degree of side chain rigidity, which affects the distribution of electrostatic charge at the substrate ***binding*** ***site*** .

L39 ANSWER 18 OF 18 CAPLUS COPYRIGHT 2002 ACS

1985:608046 Document No. 103:208046 Production of 2-keto-L-gulonate, an intermediate in L-ascorbate synthesis, by a genetically modified Erwinia herbicola. Anderson, Stephen; Marks, Cara Berman; Lazarus, Robert; Miller, Jeffrey; Stafford, Kevin; Seymour, Jana; Light, David; Rastetter, William; ***Estell, David*** (Dep. Biocatal., Genentech, Inc., South San Francisco, CA, 94080, USA). Science (Washington, D. C., 1883-), 230(4722), 144-9 (English) 1985. CODEN: SCIEAS. ISSN: 0036-8075.

AB A new metabolic pathway was created in E. herbicola that gives it the ability to produce 2-keto-L-gulonic acid [526-98-7], an important intermediate in the synthesis of L-ascorbic acid. Initially, a Corynebacterium ***enzyme*** that could stereoselectively reduce 2,5-diketo-D-gluconic acid to 2-keto-L-gulonic acid was identified and purified. DNA probes based on amino acid sequence information from 2,5-diketo-D-gluconic acid reductase [95725-95-4] were then used to isolate the gene for this ***enzyme*** from a Corynebacterium genomic library. The 2,5-diketo-D-gluconic acid reductase-coding region was fused to the Escherichia coli trp promoter and a synthetic ribosome-***binding*** ***site*** and was then introduced into E. herbicola on a multicopy plasmid. E. herbicola Naturally produces 2,5-diketo-D-gluconic acid via glucose oxidn., and when recombinant cells expressing the plasmid-endcoded reductase were grown in the presence of glucose, 2-keto-L-gulonic acid was made and released into the culture medium. Thus, it is possible to create novel in vivo routes for the synthesis of important specialty chems. by combining useful metabolic traits from diverse sources in a single organism.

=> S L36 AND L1
L40 3824 L36 AND L1

=> S L36 AND L13
L41 168 L36 AND L13

=> S L41 AND L4
L42 8 L41 AND L4

=> S L42 NOT (L11,L21,L39)
L43 8 L42 NOT ((L11 OR L21 OR L39))

=> D 1-8 CBIB ABS

L43 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2002 ACS
2002:90104 Document No. 136:130550 Zinc finger domain recognition code for use in designing DNA binding proteins. Sera, Takashi (Syngenta Participations A.-G., Switz.). PCT Int. Appl. WO 2002008286 A2 20020131, 143 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-EP8367 20010719. PRIORITY: US 2000-PV220060 20000721.
AB The present invention relates to DNA binding proteins comprising zinc finger domains in which two histidine and two ***cysteine*** residues coordinate a central zinc ion. More particularly, the invention relates to the identification of a context-independent recognition code to design zinc finger domains. This code permits identification of an amino acid for positions -1, 2, 3 and 6 of the .alpha.-helical region of the zinc finger domain from four-base pair nucleotide target sequences. The invention includes zinc finger proteins (ZFPs) designed using this recognition code, nucleic acids encoding these ZFPs and methods of using such ZFPs to modulate gene expression, alter genome structure, inhibit viral replication and detect alterations (e.g., nucleotide substitutions, deletions or insertions) in the ***binding*** ***sites*** for such proteins. The invention may also be used for target-specific introduction of exogenous genes into genome of an organism or excision of endogenous genes from genome using transposases. In addn., the invention provides a rapid method of assembling a ZFP with three or more zinc finger domains using three sets of 256 oligonucleotides, where each set is designed to target the 256 different 4-base pair targets and allow prodn. of all possible 3-finger ZFPs (i.e., >>106) from a total of 768 oligonucleotides. A method of synthesizing a nucleic acid encoding 3 zinc finger domains, sepd. by no more than 10 amino acids is provided. Three double-stranded oligonucleotides encoding each of the 3 zinc finger domains are amplified using PCR wherein the primers to the first and third oligonucleotide include a restriction endonuclease recognition site, which when cleaved by the restriction ***enzyme*** generate complementary ends that may be ligated to produce a nucleotide contg. 3 zinc finger domains. Similar methods can be used to generate nucleic acids encoding 4 or more zinc finger domains. Fusion proteins contg. ZFPs and transcriptional regulatory or DNA binding proteins are another embodiment of the invention.

L43 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2002 ACS
2001:453251 Document No. 135:56056 ***Targeting*** of growth factor processing by suppression of synthesis of an endosomal ***cysteine*** proteinase as anti-cancer therapy. Brodt, Pnina; Navab, Roya (McGill University, Can.). PCT Int. Appl. WO 2001044464 A1 20010621, 53 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-CA1460 20001206. PRIORITY: US 1999-PV170777 19991215; US 2000-PV233484 20000919.
AB The present invention relates to ***targeting*** of growth factor processing for the prevention of tumor cell proliferation and/or for the induction of tumor cell apoptosis or the spontaneously "collapsing" (suicidal) tumors and therapeutical methods thereof. More precisely, the

present invention relates to an anti-cancer compd. for preventing tumor cell proliferation and/or inducing tumor cell apoptosis, which comprises a compd. specifically targeted directly or indirectly at an endosomal ***enzyme*** involved in cellular processing of a growth factor, regulation of growth factor mediated signaling and tumorigenicity. Preferred anti-cancer compd. comprising ***cysteine*** proteinase inhibitors and antisense of cathepsin mRNA. There is demonstrated that suppression of synthesis of an endosomal ***cysteine*** proteinase can lead to a redn. in growth factor ***binding*** ***sites*** and to a loss of the tumor ability to proliferate and invade.

L43 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2002 ACS

2001:292110 Document No. 136:113417 Characterization of two genes encoding the mitochondrial alternative oxidase in Chlamydomonas reinhardtii. Dinant, Monique; Baurain, Denis; Coosemans, Nadine; Joris, Bernard; Matagne, Rene F. (Genetique des Microorganismes, Departement de Biologie Vegetale, Universite de Liege, Liege, Sart Tilman, 4000, Belg.). Current Genetics, 39(2), 101-108 (English) 2001. CODEN: CUGED5. ISSN: 0172-8083. Publisher: Springer-Verlag.

AB Two cDNA clones (AOX1 and AOX2) and the corresponding genes encoding the alternative oxidases (AOXs) from Chlamydomonas reinhardtii were isolated and sequenced. The cDNAs, AOX1 and AOX2, contained open reading frames (ORFs) encoding putative proteins of 360 amino acids and 347 amino acids, resp. For each of the ORFs, a potential mitochondrial- ***targeting*** sequence was found in the 5'-end regions. In comparison to AOX ***enzymes*** from plants and fungi, the predicted amino acid sequences of the ORFs showed their highest degree of identity with proteins from Aspergillus niger (38.1% and 37.2%) and Ajellomyces capsulatus (37% and 34.9%). Several residues supposed either to be Fe ligands or to be involved in the ubiquinol- ***binding*** ***site*** were fully conserved in both C. reinhardtii putative AOX proteins. In contrast, a ***cysteine*** residue conserved in the sequences of all higher plants and probably involved in the regulation of the ***enzyme*** activity was missing both from the AOX1 and AOX2 amino acid sequences and from protein sequences from various other microorganisms. The transcriptional expression of the AOX1 and AOX2 genes in wild-type cells and in mutant cells deficient in mitochondrial complex III activity was also investigated.

L43 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2002 ACS

2000:255784 Document No. 133:116637 Deletion of the parasite-specific insertions and mutation of the catalytic triad in glutathione reductase from chloroquine-sensitive Plasmodium falciparum 3D7. Gilberger, T.-W.; Schirmer, R. H.; Walter, R. D.; Muller, S. (Bernhard Nocht Institute for Tropical Medicine, Biochemical Parasitology, Hamburg, D-20359, Germany). Molecular and Biochemical Parasitology, 107(2), 169-179 (English) 2000. CODEN: MBIPDP. ISSN: 0166-6851. Publisher: Elsevier Science Ireland Ltd..

AB The flavoenzyme glutathione reductase (GR; NADPH + glutathione disulfide + H+.fwdarw. NADP++ 2 glutathione-SH) of Plasmodium falciparum is a promising drug target against tropical malaria. As P. falciparum genes are assumed to be highly polymorphic we have cloned and expressed the GR cDNA of the chloroquine-sensitive strain 3D7. In comparison to the known GR of the chloroquine-resistant K1 strain there are three base exchanges all of them leading to amino acid substitutions (residues 281, 285 and 335). The catalytic efficiency kcat/Km of the 3D7 ***enzyme*** is 5-fold lower than for the K1 ***enzyme*** . In contrast, vis-a-vis the drugs carmustine, methylene blue and fluorophenyliso-alloxazine the two ***enzyme*** species exhibited identical inhibition kinetics. Two structural motifs which are specific for P. falciparum GR were studied by mutational deletion anal. of 3D7 GR. Loop 126-138 appears to be important for folding and stability of the ***enzyme*** , whereas the subdomain 318-350 was found to be involved in FAD-binding. The subdomain has no major influence on the known functions of the catalytic triad Cys-40, Cys-45 and His-485'. Flavin absorption spectroscopy of inactive point mutants showed that Cys-45 forms a thiolate charge transfer complex and Cys-40 is the interchange thiol, which reduces glutathione disulfide. The mutant His-485 Gln had a normal Km for glutathione disulfide redn. but only 0.8% residual catalytic activity when compared with wild-type GR, which confirms its function as an acid/base catalyst. The parasite-specific domains in combination with the reactive catalytic

residues appear to be a suitable target matrix for inhibiting GR in vivo.

L43 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2002 ACS
1997:299880 Document No. 126:340419 Site-directed mutagenesis of the yeast V-ATPase A subunit. Liu, Qing; Leng, Xing-Hong; Newman, Paul R.; Vasilyeva, Elena; Kane, Patricia M.; Forgac, Michael (Dep. Cellular Mol. Physiol., Tufts Univ. School Med., Boston, MA, 02111, USA). J. Biol. Chem., 272(18), 11750-11756 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB To investigate the function of residues at the catalytic nucleotide ***binding*** ***site*** of the V-ATPase, we have carried out site-directed mutagenesis of the VMA1 gene encoding the A subunit of the V-ATPase in yeast. Of the three ***cysteine*** residues that are conserved in all A subunits sequenced thus far, two (Cys284 and Cys539) appear essential for correct folding or stability of the A subunit. Mutation of the third ***cysteine*** (Cys261), located in the glycine-rich loop, to valine, generated an ***enzyme*** that was fully active but resistant to inhibition by N-ethylmalemide, 7-chloro-4-nitrobenz-2-oxa-1,3-diazole, and oxidn. To test the role of disulfide bond formation in regulation of vacuolar acidification in vivo, we have also detd. the effect of the C261V mutant on ***targeting*** and processing of the sol. vacuolar protein carboxypeptidase Y. No difference in carboxypeptidase Y ***targeting*** or processing is obsd. between the wild type and C261V mutant, suggesting that disulfide bond formation in the V-ATPase A subunit is not essential for controlling vacuolar acidification in the Golgi. In addn., find phase endocytosis of Lucifer Yellow, quinacrine staining of acidic intracellular compartments and cell growth are indistinguishable in the C261V and wild type cells. Mutation of G250D in the glycine-rich loop also resulted in destabilization of the A subunit, whereas mutation of the lysine residue in this region (K263Q) gave a V-ATPase complex which showed normal levels of A subunit on the vacuolar membrane but was unstable to detergent solubilization and isolation and was totally lacking in V-ATPase activity. By contrasts, mutation of the acidic residue, which has been postulated to play a direct catalytic role in the homologous F-ATPases (E286Q), had no effect on stability or assembly of the V-ATPase complex, but also led to complete loss of V-ATPase activity. The E286Q mutant showed labeling by 2-azido-[32P]ATP that was approx. 60% of that obsd. for wild type, suggesting that mutation of this glutamic acid residue affected primarily ATP hydrolysis rather than nucleotide binding.

L43 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2002 ACS
1996:590564 Document No. 125:241581 Biotin-labeled peptidyl diazomethane inhibitors derived from the substrate-like sequence of cystatin: ***targeting*** of the active site of cruzipain, the major ***cysteine*** proteinase of Trypanosoma cruzi. Lalmanach, Gilles; Mayer, Roger; Serveau, Carole; Scharfstein, Julio; Gauthier, Francis (Fac. Med., Univ. Francois Rabelais, Tours, 37032, Fr.). Biochem. J., 318(2), 395-399 (English) 1996. CODEN: BIJOAK. ISSN: 0264-6021.
AB Biotin-labeled peptidyl diazomethane inhibitors of ***cysteine*** proteinases, based on the N-terminal substrate-like segment of human cystatin C, a natural inhibitor of ***cysteine*** proteinases, were synthesized. These synthetic derivs. were tested as irreversible inhibitors of cruzipain, the major ***cysteine*** proteinase of Trypanosoma cruzi, to compare the kinetics of the inhibition of the parasite proteinase with that of the mammalian cathepsins B and L. The accessibility of the active sites of these proteinases to these probes was also investigated. The inhibition of cruzipain by Biot-LVG-CHN2 (where Biot represents biotinyl and L, V and G are single-letter amino acid residue abbreviations) and Biot-Ahx-LVG-CHN2 (where Ahx represents 6-aminohexanoic acid) was similar to that of unlabeled inhibitor. Biotin labeling of the inhibitor slowed the inhibition of both cathepsin B and cathepsin L. Adding a spacer arm (Ahx) between the biotin and the peptide moiety of the deriv. increased the inhibition of cathepsin B but not that of cathepsin L. The discrimination provided by this spacer is probably due to differences in the topologies of the ***binding*** ***sites*** of proteinases, a feature that can be exploited to improve ***targeting*** of individual ***cysteine*** proteinases. Anal. of the blotted proteinases revealed marked differences in the accessibility of extravidin-peroxidase conjugate to the proteinase-bound biotinylated

inhibitor. Cruzipain mols. exposed to Biot-LVG-CHN2 or Biot-Ahx-LVG-CHN2 were readily identified, but the reaction was much stronger when the ***enzyme*** was treated with the spacer-contg. inhibitor. In contrast with the parasite ***enzyme*** , rat cathepsin B and cathepsin L treated with either Biot-LVG-CHN2 or Biot-Ahx-LVG-CHN2 produced no detectable bands. Papain, the archetype of this family of proteinases, was poorly labeled with Biot-LVG-CHN2, but strong staining was obtained with Biot-Ahx-LVG-CHN2. These findings suggest that optimized biotinylated diazomethanes might considerably improve their selectivity for the T. cruzi target ***enzyme*** .

L43 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2002 ACS

1991:204583 Document No. 114:204583 Differential regulation of mannose 6-phosphate receptors and their ligands during the myogenic development of C2 cells. Szebenyi, Gyorgyi; Rotwein, Peter (Sch. Med., Washington Univ., St. Louis, MO, 63110, USA). J. Biol. Chem., 266(9), 5534-9 (English) 1991. CODEN: JBCHA3. ISSN: 0021-9258.

AB The mammalian insulin-like growth factor II/cation-independent mannose 6-phosphate receptor (IGF-II/CIMPR) mediates both ***targeting*** and endocytosis of mannose 6-phosphate-contg. proteins and binds insulin-like growth factor II (IGF-II). The cation-dependent mannose 6-phosphate receptor (CDMPR) lacks an IGF-II- ***binding*** ***site*** and participates only in the intracellular trafficking of lysosomal ***enzymes*** . During terminal differentiation of the myogenic C2 cell line, there is an increase in cell surface expression of the IGF-II/CIMPR in parallel with a rise in secretion of IGF-II. In this study IGF-II/CIMPR mRNA increases by >10-fold during the initial 48 h of C2 muscle differentiation with kinetics similar to the rise in IGF-II mRNA. Comparable levels of both mRNAs are expressed in C2 myotubes and in primary cultures of fetal muscle. By contrast, no change is obsd. in CDMPR transcript abundance during differentiation, and only a small, transient increase is seen in the enzymic activities and mRNA levels of several lysosomal ***enzymes*** . The differential regulation of the 2 mannose 6-phosphate receptors during muscle differentiation suggests that they may serve distinct functions in development.

L43 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2002 ACS

1991:139411 Document No. 114:139411 Conjugated ***targeting*** polypeptides and methods for their preparation and use. Schultz, Peter (University of California, Oakland, USA). PCT Int. Appl. WO 9005749 A1 19900531, 67 pp. DESIGNATED STATES: W: AU, DK, FI, JP, KR, SU; RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1989-US5257 19891115. PRIORITY: US 1988-273786 19881118.

AB Polypeptides having active functionalities (e.g. reporter mols. or chemotherapeutic agents) bound proximate a ***binding*** ***site*** are prepd. by (a) combining the polypeptides with ligands capable of binding to the ***binding*** ***site*** , the ligands having a reactive group cleavably attached; (b) attaching the reactive group to an amino acid side chain on the polypeptide; (c) cleaving the reactive group from the ligand, whereby a moiety of the reactive group remains bound to the polypeptide through the amino acid side chain; and (d) removing the ligand from the polypeptide. This moiety may itself be the active functionality or may be the attachment site for joining the active functionality. Synthetic polypeptides may be prepd. having a rare or nonnatural amino acid proximate the ***binding*** ***site*** which may be used to attach the active functionality. The Fab fragment of IgA MOPC315, which binds substituted 2,4-dinitrophenyl (DNP) group-contg. ligands, was affinity labeled by reaction with (N-2,4-dinitrophenyl)-2-aminoethyl 4-oxobutyl disulfide (prepn. given) and NaCNBH3, cleavage with dithiothreitol, reaction with 2,2'-dithiopyridine, and disulfide exchange with N-fluoresceinthioureido-2-mercaptoethylamine. The resulting adduct was isolated in 84% overall yield, with >90% incorporation of the fluorophore. Addn. of ligand DNP-glycine to the fluorescein-Fab adduct resulted in a decrease in fluorescence, providing a direct assay of ligand binding. The assocn. const. of DNP-glycine to the adduct was 3.0 .times. 105 M-1, almost the same as that for the underivatized antibody (2.0 .times. 105 M-1).